

Date of issue: 04.12.1996
Reference: R 102/100
Version: 1.0

6. Test Procedure

Bulk- Material

A prerequisite for the use of this analytical method is that the tests are carried out in a laboratory which is well equipped for analysis. It is also necessary that the analyst is suitably qualified, is familiar with the safe handling of chemicals and analytical apparatus, has been informed about general laboratory guidelines relating to safety precautions and accident prevention, and strictly observes these guidelines.

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1. Appearance

Assess the appearance of 10 ml of solution in a colourless test tube with an I.D. of 1.6 cm by daylight against a white background. To test the odour, put a little solution on a filter paper. The appearance and odour must correspond to the reference sample.

2. Identity (HPTLC)

Principle

Use the test and control solutions described under Item 5 to test by thin-layer chromatography on HPTLC plates. Evaluate the results visually by daylight.

Equipment

- 50 ml volumetric flasks
- 2 ml volumetric pipettes
- 10 x 10 cm ready-made HPTLC plates, silica gel 60 F₂₅₄ (Merck Co.)
- Application device: Linomat IV (Camag Co.)
- Horizontal developing chamber (Camag Co.)

Reagents

- KBR 3023 reference standard sample
- Methanol p.a.
- Cyclohexane p.a.
- Acetic acid ethyl ester p.a.
- Potassium permanganate (0.1 %)

Test solution

Use test solution Item 5.

Control solution I

Treat 625 mg control sample AUTAN LOTION 20% WB R102/100 as described under "Test solution" (Item 5).

Control solution II

Use control solution Item 5.

HPTLC conditions

Carrier material	HPTLC plates, silica gel 60 F ₂₅₄ (10 x 10 cm) (Merck Co., Darmstadt)
Developing solvent	Cyclohexane - acetic acid ethyl ester (V/V) 6 : 4
Chamber saturation	Without chamber saturation
Volume applied	5 µl per sample

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Mode of application In stripes 1 cm wide, with Linomat IV
 Length of run 4 cm

Method

Once the spots of the substance have dried, develop the plate in the horizontal developing chamber.

Evaluation

After drying put the plate two seconds into a dipping tank filled with potassium permanganate (0.1%).

Identification of the product

After 10 - 15 mins light spots are visible. To identify the product, compare the distribution of the zones for the various substances in the test and control solutions. The separated substance zones obtained for the test and control solutions must correspond in size, intensity and Rf value.

KBR 3023	<u>Rf value</u>
	0.3

3. pH value

Potentiometric determination according to European Pharmacopoeia. Direct measurement with an emulsion electrode.

4. Refractive index

According to European Pharmacopoeia.

5. Assay (HPLC)

5.1. KBR 3023

Principle

Dissolve the sample with methanol. Determine the content of relevant component by high pressure liquid chromatography (HPLC).

Equipment

- 50 ml volumetric flasks
- 2 ml volumetric pipettes
- Pasteur pipettes, sampling jars
- HPLC with detector (UV range, variable wavelength), sampler and evaluation unit (e.g. Shimadzu LC-6A with CR-4A)

Reagents

- KBR 3023 reference standard substance
- HPLC-grade methanol
- Acetonitrile (LiChrosolv)
- HPLC-grade distilled water
- Tetrahydrofuran (LiChrosolv)

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Test solution

Place approx. 625 mg sample in a 50 ml volumetric flask (weigh accurately), top up to calibration mark with methanol and shake well.

Control solution

Dissolve approx. 125 mg KBR 3023 reference standard (weigh accurately) with methanol in a 50 ml volumetric flask, top up to calibration mark with methanol and shake well.

HPLC conditions

Column	Metal column made of V ₂ A steel, 25 cm long, internal diameter 4 mm. Filled with LiChrosorb RP 8, 7 µm (e.g. Hibar column, Merck Co., Darmstadt)
Eluent	Acetonitrile - tetrahydrofuran - water (V/V) 30 : 10 : 60
Flow rate	1.5 ml/min
Temperature	Room temperature
Injection volume	20 µl
Detection	UV 210 nm
Retention time	KBR 3023: approx. 5.0 mins
Analysis time	7 mins

Notes

The conditions given above are intended as a guide. To obtain optimum results they may have to be adapted to suit the available technical conditions and the properties of the column.

Method

External standard method with area evaluation

Chromatography is performed with the test and control solutions under the conditions specified. The retention time of the chromatograms of the test and control solutions must be the same.

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Calculation

$$G = \frac{P_p \times W_v \times G_v}{P_v \times W_p}$$

- G = KBR 3023 content in g/100 g
G_v = KBR 3023 content of reference standard substance in %
P_p = KBR 3023 peak surface area of test solution
P_v = KBR 3023 peak surface area of control solution
W_v = Weight of KBR 3023 reference standard substance in mg
W_p = Weight of sample in mg

6. Evaluation of batch

- 6.1. Test findings
6.2. In-process control data
6.3. Documentation of production plants